REMARKS

Upon entry of this amendment, claims 1, 101, 102, 182, and 189-210 are pending in the instant application. Claims 1, 101, 102 and 182 have been amended and claims 197-210 have been added. Claims 5-12, 14-17, 19, 20, 77-79, 81-85, 88, 90-94, 103-110, 112-114, 116-120, 122-127, 130-137, 139-148, 150-159, 161-174, 176-181, and 183-188 have been cancelled without prejudice or disclaimer.

Support for the claim amendments presented herein is found throughout the specification and in the claims as originally filed. Support for the double mutant T7 RNA polymerases recited by amended claims 1, 101, 102 and 182, is found at least in paragraphs **[0050]**, **[0071]**, **[00114]**, **[00115]**, **[00118]**, **[00122]**, **[00181]**, and **[00213]**, and in claims 8 and 40 as originally filed. New claims 197-210 have been added solely to streamline the claims that depend from independent claims 1, 101, 102 and/or 182. Support for these new claims is found throughout the specification and in the claims as originally filed. For example, support for new claims 197-200 is found at least in paragraphs **[0038]** through **[0044]**, **[0051]**, **[0072]**, **[00118]**, and **[00122]**, and in claims 9-11 and 41-43 as originally filed. Support for new claims 201-203 is found at least in paragraphs **[0053]**, **[0074]** and **[00120]**. Support for new claims 204-206 is found at least in paragraphs **[0036]**, **[0037]**, **[0055]**, **[0056]**, **[0076]**, **[0077]**, **[00121]**, **[00122]** and **[00213]** and in claims 18-20 and 50-52 as originally filed. Support for new claim 207 is found at least in paragraphs **[0068]** and **[00133]** and in claim 30 as originally filed. Support for new claim 208 is found at least in paragraphs **[0122]** and **[00140]**, and support for new claim 209 is found at least in paragraphs **[0052]**, **[0073]** and in claims 13 and 45 as originally filed. Finally, support for new claim 210 is found at least in paragraphs **[00122]** and **[00213]**.

In addition, the specification has been amended at paragraphs **[00115]** and **[00118]** to correct an inadvertent typographical error. Accordingly, the present amendments are fully supported by the original disclosure, and no new matter has been added.

Claim Rejections Under 35 U.S.C. § 112

Claims 6, 7, 104 and 105 have been rejected under 35 U.S.C. § 112, first paragraph, for lack of written description. Claims 6, 7, 104 and 105 have been cancelled herein, thereby obviating all rejections of these claims.

Claims 1, 5-12, 14-17, 19, 20, 77-79, 81-85, 88, 90-94, 101-103, 108-100, 112-114, 116-120, 122-127, 130-137, 139-148, 150-156, 159, 159 [sic], 161-174, 176-190, 195 and 196 have been rejected under 35 U.S.C. § 112, first paragraph for lack of written description. According to the Examiner on page 10 of the Office Action, "while one of ordinary skill in the art would have been able to perform the methods with the Y639F/H784A double mutant T7 RNA polymerase, applicant clearly has not shown that any other modified RNA polymerase would function as claimed."

The pending independent claims have been amended herein to recite methods that use a double-mutant T7 RNA polymerase that consists of a Y639F/H784A mutant T7 RNA polymerase in which the tyrosine residue at position 639 has been changed to a phenylalanine and the histidine residue at position 784 has been changed to an alanine. Thus, the independent claims have been amended to include the subject matter of previous claims 8, 106 and 157, which claims were not included in this written description rejection. As acknowledged by the Examiner on pages 7, 8 and 10 of the Office Action, the specification as originally filed provides sufficient written description for the methods recited by the amended claims presented herein. Accordingly, withdrawal of this rejection is requested.

Claim Rejections Under 35 U.S.C. § 103

Claims 1, 5-7, 9-12, 14-16, 19-20, 77-79, 81-85, 88, 90-94, 101-105, 107-109, 112-114, 116-120, 122-127, 133-136, 139-147, 150-155, 158, 161-173 and 176-190 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,660,985 by Pieken et al. ("Pieken") in view of U.S. Patent No. 5,914,396 by Cook et al. ("Cook"), Brieba et al., Biochemistry, vol. 39:919-923 (2000) ("Brieba"), U.S. Patent No. 6,107,037 by Sousa et al. ("Sousa"), Bishop et al., J. Virol., vol. 8(1):66-73 (1971) ("Bishop"), and Chow et al., Proc. Nat. Acad. Sci. USA, vol. 68(4): 752-756 (1971) ("Chow"). Claims 17, 110, 130-132, 137, 148, 159 and 174 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Pieken in view

Brieba, Sousa, Bishop, Chow and Milligan et al., Methods Enzymol., vol. 180: 51-62 (1989) ("Milligan"). Claims 8, 106, 157, 195 and 196 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Pieken in view Brieba, Sousa, Bishop, Chow and Padilla et al., Nucleic Acids Research, vol. 30(24): e138, pages 1-4 (2002) ("Padilla").

As an initial matter, Applicants note that the Examiner listed the Cook reference in the first paragraph of the obviousness rejection of claims 1, 5-7, 9-12, 14-16, 19-20, 77-79, 81-85, 88, 90-94, 101-105, 107-109, 112-114, 116-120, 122-127, 133-136, 139-147, 150-155, 158, 161-173 and 176-190 on page 11 of the Office Action, but did not specifically address this reference in the arguments supporting this rejection. In addition, the Cook reference was not cited in the Notice of References Cited by the Examiner. Applicants assume that the Cook reference was inadvertently included in this rejection and have not addressed Cook in the remarks set forth below. The Examiner is asked to contact the undersigned if this understanding is incorrect.

The methods recited by the amended claims presented herein were carefully selected to produce efficient manufacturing processes that generate stabilized oligonucleotide transcripts and/or aptamers of a sufficient length in a commercially effective manner using a double-mutant T7 RNA polymerase that consists of a Y639F/H784A mutant T7 RNA polymerase in which the tyrosine residue at position 639 has been changed to a phenylalanine and the histidine residue at position 784 has been changed to an alanine.

As Applicant has repeatedly demonstrated in previous responses, the cited references, alone or in combination, do not disclose or suggest the claimed methods using the Y639F/H784A double-mutant T7 RNA polymerase.

Briefly, Pieken describes only wild-type T7 RNA polymerase, does not disclose any transcription reaction mixtures that include both magnesium and manganese ions, and explicitly acknowledges that the wild-type T7 RNA polymerase does not recognize bulkier 2'-substituted NTPs, such as 2'-OMe. (*See* Pieken, col. 8, lines 26-29). Brieba does not disclose any mutant RNA polymerases that can incorporate bulkier 2'-modified NTPs such as 2'-OMe NTPs, nor any transcription reaction mixtures that include both magnesium and manganese ions. Likewise, Sousa does not teach or suggest any mutant RNA polymerases that can incorporate bulkier 2'-modified NTPs, nor does this reference disclose the use of both magnesium and manganese ions in a single transcription reaction mixture. Milligan does not teach or suggest the use of any

mutant RNA polymerases, let alone mutant RNA polymerases that can incorporate bulkier 2'-modified NTPs such as 2'-OMe NTPs, nor does this reference disclose the use of both magnesium and manganese ions in a single transcription reaction mixture. Bishop does not teach or suggest the use of any mutant RNA polymerases and only describes the transcription reaction conditions for one particular unmodified RNA polymerase.

In fact, no combination of these references describes methods that use the double-mutant T7 RNA polymerase Y639F/H784A in conjunction with a transcription reaction mixture that includes both magnesium and manganese ions, wherein the concentration of magnesium ions is about 3 to 5 times greater than the concentration of manganese ions, to successfully produce stabilized nucleic acids having a length in the range of 30-50 nucleotides.

The addition of the Chow reference in these rejections fails to remedy the deficiencies in the Pieken, Brieba, Sousa, Bishop and/or Milligan references. In fact, the teachings of the Chow reference simply underscores Applicants' argument that each polymerase, wild-type or mutated, has a specific set of transcription reaction conditions that promote efficient and effective transcription of a given oligonucleotide template. Figure 2 of the Chow reference demonstrates that the conditions for the RNA polymerase in influenza virions are not suitable for the RNA polymerase in vesicular stomatitis virus (VSV), as transcription activity in the VSV was actually inhibited by the presence of manganese ions. (*See also*, Chow at pg. 753, col. 1, 1st full para.).

Contrary to the Examiner's assertions, Chow does not teach the combination of magnesium and manganese ions is generally useful for optimal performance of any polymerase, let alone a mutated T7 RNA polymerase such as the Y639F/H784A specific double-mutant recited by the amended claims. In contrast, this reference is evidence that even within a single class of polymerases, *e.g.*, viral RNA polymerases, each individual polymerase can act in a distinctly different manner under the same conditions, and furthermore, each polymerase has an individual set of basic requirements for initiating transcription generally and reaching a maximal level of transcription. As such, transcription conditions that are useful for one type of polymerase cannot be used to predict the transcription conditions that will be needed for another polymerase to successfully and efficiently produce nucleic acid transcripts.

Accordingly, there is no teaching or other objective evidence that the conditions described by Chow would be broadly applicable to other types of non-viral RNA polymerases.

Thus, there is no reason that the skilled artisan seeking to use a mutant RNA polymerase to produce stabilized nucleic acid molecules by incorporating 2'-OMe NTPs within an oligonucleotide transcript would look to Chow for teachings about polymerases in general, let alone for guidance regarding the conditions that would be necessary and sufficient to allow the Y639F/H784A double-mutant T7 RNA polymerase to incorporate bulkier 2' substituents into oligonucleotide transcripts.

The data and information presented in the cited references demonstrate that varying the composition of the transcription reaction mixture, such as for example, by varying the concentration of magnesium and manganese ions, can dramatically affect transcription yield. As such, the effect(s) of varying the ion concentrations or other reaction mixture conditions on transcription could not have been predicted based on the teachings in the art at the time of instant invention. The identification of the ideal composition of the transcription reaction mixture, as well as the ideal ratio of manganese and magnesium ions, is not a "mere optimization" of ranges, nor would the ideal transcription conditions have been obvious in view of the state of the art at the time of the instant invention.

The Examiner's reliance on whether the presence of both magnesium and manganese ions in transcription reaction conditions was known in the art at the time the instant application was filed is misplaced. The methods provided herein were carefully selected to produce efficient manufacturing processes that generate stabilized oligonucleotide transcripts and/or aptamers in a commercially effective manner. The transcription reaction mixture and conditions recited by the amended claims, particularly the use of both magnesium and manganese ions, allow the modified RNA polymerases to accept <u>any</u> of the 2'-OMe NTPs as substrates and incorporate these modified nucleotides into the transcript during both the initiation and elongation portions of transcription.

Prior to the methods disclosed in the instant application, the skilled artisan could not incorporate 2'-OMe GTP in an oligonucleotide transcript -- none of the cited references, not even Padilla, disclose methods in which a polymerase is able to incorporate 2'-OMe GTP into an oligonucleotide transcript. Those of ordinary skill in the art at the time of the instant invention would have recognized the difficulty in incorporating 2'-OMe GTP during oligonucleotide transcription. This continued failure of others in the field to generate oligonucleotide transcripts

that include 2'-OMe GTP is further evidence that the transcription conditions recited in the amended claims presented herein were not obvious at the time of filing.

Moreover, the methods of the claimed invention are a significant improvement over prior methods of producing stabilized nucleic acid molecules, such as stabilized aptamers. The methods provided herein do not require post-translational modifications to produce stabilized aptamers. Prior to the instant invention, 2'-OMe NTPs were incorporated within a given aptamer sequence by replacing the 2'-substituent of a nucleotide with a 2'-OMe group, a process referred to as "post-SELEX™ modification." Once the 2'-OMe substituent(s) was incorporated post-translationally, the skilled artisan would then have to re-evaluate the post-translationally stabilized aptamer to determine whether the modification would negatively affect or otherwise impact binding of the stabilized aptamer to the target molecule.

In contrast, the methods of the invention generate oligonucleotides that (i) are already stabilized by virtue of including one or more 2'-OMe NTPs within the transcribed nucleotide sequence and (ii) are already known to bind the desired target because only the stabilized transcribed nucleotide sequences that bind to the target are retained in the claimed methods. Thus, the manufacturing methods provided herein, which require fewer steps than post-SELEX™ modification and provide a commercially efficient and effective method for producing stabilized aptamers, are a significant improvement over previous methods of manufacturing stabilized oligonucleotides known in the art at the time of the instant invention.

Applicants submit that there is no objective reason or other evidence provided in the cited references, alone or in combination, that the success of the claimed methods and transcription conditions would have been predictable, particularly in light of the inability of others in the field, even after the filing of the instant application, to identify and successfully use a mutant polymerase to incorporate all 2'-OMe NTPs, including 2'-OMe GTP, within stabilized aptamers and/or stabilized oligonucleotide transcripts. Thus, any suggestion that it would have been obvious to use the double-mutant T7 RNA polymerase, transcription reaction mixtures and conditions recited by the amended claims is an improper application of hindsight based on Applicants' disclosure in the instant application. Accordingly, Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness and request that these rejections be withdrawn.

CONCLUSION

On the basis of the foregoing amendments, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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